Protection from Carcinogen-induced Murine Bladder Carcinoma by Interferons and an Oral Interferon-inducing Pyrimidinone, Bropirimine

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ABSTRACT

Interferons (IFNs) have established activities as antivirals and inhibitors of viral and transplantable tumors. To establish whether IFNs or their inducers can affect induction of carcinogenesis in vivo, the bladder-specific carcinogen N-(4-(5-nitro-2-furyl)-2-thiazolyl)formamide (FANFT) was administered in the diet at 0.11 or 0.13% (w/w) to female C3H/He mice beginning at 7 weeks of age. Mice treated with the IFN-inducing bropirimine [2-amino-5-bromo-6-(4-hydroxy-2-thiazolyl)pyrimidinone] i.p. twice a week for 14 weeks starting on day 30 of start of FANFT feeding developed fewer transitional cell carcinomas (TCC) than mice treated with the vehicle. Bropirimine (200 mg/kg twice a week) orally resulted in even greater effectiveness: 6 of 43 bladders with TCC for bropirimine-treated mice versus 24 of 39 for control glycine buffer-treated mice (P < 0.01, x2 test). Mice treated i.p. daily on days 29 through 210 with 5,000 units of IFN (specific activity, 2.0 x 10^7 units/mg) had 0 of 15 TCC while control mice had 7 of 13 TCC (P < 0.001). Bladders of untreated mice were also significantly heavier than those of IFN-treated or bropirimine-treated mice. This dose of IFN treatment was confirmed as effective in a second experiment, in which mice were treated daily on days 30-223 with 5,000 units of IFN (specific activity, 1.2 x 10^7 units/mg). This resulted in 4 of 25 bladders with TCC versus 24 of 39 for control mice (P < 0.001). A higher dose of IFN (50,000 units of IFN interferon daily) was toxic; 24 of 30 mice died within 2 months. IFN and IFN inducer, bropirimine, inhibited development and progression of FANFT-induced bladder TCC in vivo and thus may have roles as chemopreventive modalities.

INTRODUCTION

Human malignancies may arise after exposure to environmental chemicals. TCC of the lower urinary tract, most of which develop in the bladder, is a well defined example of carcinogen-induced human malignancy. These have occurred in humans in association with exposure to arylamines such as 2-naphthylamine, benzidine, and 4-aminobiphenyl (1). Models involving carcinogen-induced murine bladder carcinomas, which mimic the pathogenesis and histology of the human disease process, have been developed (2, 3). Several carcinogens have been employed, including FANFT, N-butyl-N-(4-hydroxybutyl)nitrosamine, and bracken fern. Continuous administration of any one of these agents results in mucosal hyperplasia within 3 weeks, followed by the appearance of superficial papillary or sessile TCC in 10–30 weeks (2, 3). Hyperplasia may be histologically reversible during the first 3–4 weeks if the carcinogen exposure is stopped; however, after that time an irreversible progression to TCC occurs (2, 3).

IFNs and their inducers have demonstrated antitumor activity for established malignancies of vertebrates including humans. Effects of IFN against transplantable tumors in mice and metastatic tumors in humans suggest that IFNs might be effective in inhibiting early evolution of the carcinogenic process. Although the mechanisms of antitumor action of IFNs has not been defined and may vary for different tumors, bladder carcinomas have proven sensitive to IFNs and inducers in both mice and humans. A transplantable TCC, derived from an FANFT-induced tumor, was inhibited by IFN inducers such as bropirimine (previously designated ABPP) (4, 5). Proliferation of human TCC cells was inhibited in culture by IFN-β, and intravesical administration of an IFN inducer or IFN-α suppressed development of human bladder papillomas or carcinoma in situ (6–8).

To test the hypothesis that IFNs or their inducers might be used as chemoprophylactic compounds, we assessed murine IFN-α/β, a highly purified mouse IFN-β, and the potent murine inducer of IFN-α bropirimine (9–11) for activity against FANFT-induced murine TCC. The quantitative nature of the murine-FANFT model, together with its similarities to the human disease, make it an excellent choice for such studies.

MATERIALS AND METHODS

Mice. Six- to seven-week-old, female, C3H/He mice purchased from Sprague-Dawley (Indianapolis, IN) were housed in suspended metal cages with no more than five mice to each cage. They were fed Wayne Rodent Blox (Continental Grain Co., Chicago, IL) and HCl-acidified (pH 2.7 to 3.0) tap water ad libitum and were placed in rooms with controlled temperature (22.2–24.4°), humidity (40%), and 12-h light-dark cycles. Two weeks after arrival, mice were individually marked and were started on FANFT feeding. Mice in different groups were weighed once every 2 weeks to assess toxicity of treatment. There was no measurable effect on body weight of treatment with IFN or bropirimine at the doses used.

Drugs. FANFT was purchased from Saber Laboratories (Morton Grove, IL). It was mixed with dextrose and powdered Wayne Lab Blox at the concentration of 0.11 or 0.13% (w/w). To prepare 10 kg food, 11 g FANFT were mixed in a mortar with 89 g dextrose and this in turn was mixed with 9.9 kg powdered Wayne Lab Blox in a mechanical food mixer. Face masks as well as rubber disposable gloves were used to minimize human exposure to FANFT. Mixed diet was stored in the cold. Mice not exposed to FANFT were provided with food consisting of 9.9 kg powdered food mixed with 90 g dextrose. Food was placed in special containers which allowed free access to the mice and at the same time kept spilling of food to a minimum. At the end of FANFT feeding, mice were returned to the regular pellet food in some experiments.

Murine IFN-β (Lot 83011) was purchased from Lee BioMolecular (San Diego, CA), with specific activity of 1.2 x 10^6 units/mg protein. IFN-α/β (Lot 85119), with specific activity of 1.2 x 10^7 units/mg protein, was also provided by the same company. IFN was dissolved in 1 ml sterile distilled water and diluted in 0.4 m glycine-HCl buffer with a final concentration of 3.5. Bropirimine was prepared and provided by the Upjohn Company (Kalamazoo, MI) (10). It was administered i.p. or by gavage in a fine suspension in a vehicle composed of 5 mg/100 ml carboxymethyl-cellulose, 4 mg/100 ml polysorbate 80, 9 mg/100 ml sodium chloride, and 9% benzyl alcohol.

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2 The abbreviations used are: TCC, transitional cell carcinoma; bropirimine, 2-amino-5-bromo-6-(4-hydroxy-2-thiazolyl)pyrimidinone; FANFT, N-(4-(5-nitro-2-furyl)-2-thiazolyl)formamide; IFN, interferon; IFN-α, α interferon; IFN-β, β interferon.
PROTECTION FROM CARCINOGENESIS BY IFN OR INDUCER

In order to monitor progression of carcinogenesis, bladders of seven untreated or vehicle-treated FANFT-fed mice were examined once every 4 weeks. Mice were killed with ether and weighed and 0.25 ml WAFR fixative (24% ethanol, 10% formaldehyde, and 2% acetic acid in water) was instilled in the bladder via transurethral catheter. Bladders were dissected out of the mice while inflated with the fixative and were placed in vials. Bladders were carefully trimmed, leaving only bladder tissue. Bladders were cut into two equal halves under a dissecting binocular microscope, making sure the cut passed through the most opaque (transformed) part of the bladder. The cut “cups” were carefully dried and weighed. They were embedded in paraffin and oriented so that the cut surface (passing through the most transformed part) would be sectioned first. Three or four sections were mounted on one slide, skipping about 20 sections after each mounted section in order to make sure most of the changes in the bladder wall were represented. Sections were stained with hematoxylin and eosin and evaluated after coding to assure blinded histopathological interpretation. Bladders were classified as: normal, undergoing noncancerous changes ranging from simple hyperplasia to small benign papillomas, or showing irreversible malignant changes including carcinoma in situ or TCC (2).

At the end of the experiment, mice were killed with ether, and bladder preparation was repeated. Mice were also examined macroscopically for any irregularities in the kidneys, liver, spleen, intestine, heart, or lung.

RESULTS

Mice fed with 0.13% FANFT for 192 days developed malignant neoplasia in 81.8% of the bladders (Group 1, Table 1). Bropirimine (200 mg/kg i.p., 2 times/week) was administered to mice 30 days after FANFT feeding was begun and continued for 98 days. This schedule resulted in substantial protection against FANFT-induced carcinogenesis: 55.6% of the bladders with TCC (Group 2) versus 81.8% in mice receiving only the vehicle used for bropirimine suspension (Group 1). This ratio in bropirimine-treated FANFT-fed mice was less than half of that of FANFT-fed vehicle-treated controls (Group 1) (P < 0.05). This result contrasted with the results of delayed treatment with bropirimine (Group 3), starting on day 150 after FANFT initiation and administered at the same dose and frequency as in mice begun on bropirimine early in the neoplastic disease process. In this late treatment schedule (Group 3), no reduction in bladder to body weight ratio or frequency of neoplasia was evident upon termination of all groups 230 days after the start of FANFT feedings.

It should be noted that treatment with bropirimine i.p. was toxic even in mice not ingesting FANFT; 20 of 30 mice died prior to termination (Group 4). Surviving mice, treated i.p. with bropirimine, did not lose weight but developed a turgid abdomen filled with ascitic fluid and deposits of unabsorbed bropirimine; histology was consistent with a regional inflammatory process.

Since p.o. bropirimine was known to be effective as an IFN inducer (9–11), an experiment was designed to determine whether bropirimine by the p.o. route might be inhibitory to FANFT carcinogenesis. Mice were treated with bropirimine by gavage 30 days after initiation of FANFT feeding, on a schedule of 200 mg/kg bropirimine twice weekly for 105 days. Upon termination at day 220 of study, a significant reduction in bladder to body weight ratio was again observed in the groups of mice treated with bropirimine. In vehicle-treated mice, the ratio was 2.15 ± 0.18, while in bropirimine-treated mice it was 1.65 ± 0.12 (P < 0.03). Treatment with bropirimine orally once a week (200 mg/kg) was ineffective (bladder to body weight ratio, 2.20 ± 0.12).

Since the initial experiments suggested a reduction in TCC with bropirimine, an additional study was initiated with p.o. bropirimine and with partially purified murine IFN-α/β given i.p. (Table 2). In this study, IFN or bropirimine was again initiated on day 30 after the start of FANFT feeding but continued for a more prolonged period, until termination at day 223 (Table 2). This resulted in a highly significant reduction in bladder weights and bladder to body weight ratio with both bropirimine (Group 2) and 5 × 10⁸ units of IFN-α/β given daily (Group 3). In both groups a substantial reduction in frequency of TCC was observed (Table 2). IFN-α/β 3 times weekly (Group 4) was also effective in significantly reducing the frequency of TCC (P < 0.01) but exhibited less reduction in bladder to body weight ratio (P < 0.17). A higher dose of IFN-α/β (Group 5) was both toxic and ineffective.

Thus both bropirimine, an IFN inducer, and IFN-α/β were effective in significantly reducing the frequency of FANFT-induced TCC. However, mortality had occurred with IFN-α/β (Group 5, Table 2) and a significant reduction in body weight (P < 0.02) had occurred in other IFN-treated groups (Group 3 versus Group 1, Table 2). To further confirm the reduction in TCC and to assess weight loss (or decreased intake) as a factor, a highly purified murine IFN-β (specific activity, 2 × 10⁹ units/mg protein) was evaluated. IFN-β was administered daily from day 30 until termination at day 210 (Table 3). All IFN-treated groups gained more weight than controls. Again 5 × 10⁸ units of IFN (Group 3) were effective in significantly reducing both bladder weights and body to bladder weight ratios and the frequency of TCC. The higher dose of 5 × 10⁵ units daily (Group 2) of the purer IFN was tolerated but again ineffective in reducing the frequency of TCC, as was a low dose of 5 × 10⁴ units daily (Group 4). However, in both the high and low dose groups, marginal reduction in bladder to body weight ratio occurred.

Table 1 Effects of early or late i.p. treatment with the IFN inducer bropirimine on carcinogenesis in the mouse bladder induced by FANFT feeding (0.13%, w/w, days 0–192)

<table>
<thead>
<tr>
<th>Group</th>
<th>FANFT Treatment</th>
<th>Starts on day</th>
<th>n start</th>
<th>n end</th>
<th>Terminal bladder wt. (mg)</th>
<th>Body wt. (g)</th>
<th>P compared to Group 1</th>
<th>Histology of bladder</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+ Vehicle</td>
<td>30</td>
<td>25</td>
<td>22</td>
<td>2.64 ± 0.67*</td>
<td>1.06 ± 0.05</td>
<td>0.05</td>
<td>Normal or benign</td>
</tr>
<tr>
<td>2</td>
<td>+ 200 mg/kg bropirimine, 2 x/wk (98 days)</td>
<td>30</td>
<td>40</td>
<td>9</td>
<td>2.09 ± 0.42</td>
<td>0.64</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>+ 200 mg/kg bropirimine, 2 x/wk (98 days)</td>
<td>150</td>
<td>40</td>
<td>19</td>
<td>0.93 ± 0.17</td>
<td>0.037</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>- 200 mg/kg bropirimine, 2 x/wk (84 days)</td>
<td>30</td>
<td>30</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SE.
**Table 2 Effects of treatment with IFN-α/β or bropirimine on carcinogenesis in the mouse bladder induced by FANFT feeding (0.11%, w/w, days 0–192)**

<table>
<thead>
<tr>
<th>Group</th>
<th>FANFT</th>
<th>Treatment*</th>
<th>n start</th>
<th>n end</th>
<th>Body (g)</th>
<th>Bladder (mg)</th>
<th>Body (g)</th>
<th>Compared to Group 1 (P)</th>
<th>Normal or benign</th>
<th>TCC % TCC</th>
<th>Compared to Group 1 (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>Glycine buffer daily</td>
<td>80</td>
<td>39</td>
<td>28.5 ± 0.44a</td>
<td>67.0 ± 2.38a</td>
<td>2.39 ± 0.12</td>
<td>&lt;0.0001</td>
<td>15</td>
<td>24</td>
<td>61.5</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>200 mg/kg bropirimine, 2 × wk orally</td>
<td>45</td>
<td>43</td>
<td>28.5 ± 0.25</td>
<td>50.6 ± 1.30</td>
<td>1.78 ± 0.05</td>
<td>0.0064</td>
<td>21</td>
<td>4</td>
<td>16.0</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>5,000 units IFN-α/β daily</td>
<td>30</td>
<td>25</td>
<td>27.0 ± 0.40</td>
<td>53.0 ± 2.30</td>
<td>1.97 ± 0.09</td>
<td>0.17</td>
<td>21</td>
<td>4</td>
<td>16.0</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>5,000 units IFN-α/β, 3 wk/wk</td>
<td>30</td>
<td>25</td>
<td>27.6 ± 0.36</td>
<td>58.8 ± 4.15</td>
<td>2.13 ± 0.15</td>
<td>&lt;0.0001</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>50,000 units IFN-α/β daily</td>
<td>30</td>
<td>6</td>
<td>26.4 ± 0.48</td>
<td>55.5 ± 6.06</td>
<td>2.11 ± 0.24</td>
<td>0.32</td>
<td>4</td>
<td>2</td>
<td>33.3</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>5,000 units IFN-α/β daily</td>
<td>20</td>
<td>14</td>
<td>29.9 ± 0.57</td>
<td>48.6 ± 0.89</td>
<td>1.63 ± 0.04</td>
<td>&lt;0.0001</td>
<td>19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>200 mg/kg bropirimine, 2 × wk orally</td>
<td>20</td>
<td>19</td>
<td>28.1 ± 0.48</td>
<td>42.0 ± 1.96</td>
<td>1.50 ± 0.07</td>
<td>&lt;0.0001</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Treatment started on day 30 of start of FANFT feeding and continued until the termination of the experiment on day 223. IFN-α/β (specific activity, 2.0 × 10⁶ units/mg protein) was administered i.p. Mean body weight of all mice at start of FANFT was 20.8 g.

**DISCUSSION**

A number of defined cellular effects of IFNs could account for the inhibitory influence of IFNs or bropirimine on FANFT-induced carcinogenesis. IFNs, as does bropirimine, augment immune effector cell function, suppression of which has been identified after chemical carcinogens (12–15). In vitro (16) and in vivo (17) angiogenesis was suppressed by IFNs. The activity of ornithine decarboxylase, a rate-limiting enzyme in carcinoinduced polyamine synthesis, has been inhibited (18, 19). Cell differentiation can be modulated and oncogene expression inhibited (20–23). Finally, direct effects on cell proliferation with slowing of the mitotic cycle have been identified (24–26). Since FANFT-induced tumors can be transplanted s.c. or intravesically at defined stages of evolution (1–3), many of these alternatives can be assessed.

Inhibitory effects of IFNs and bropirimine on carcinoinduced TCC extend earlier observations on effects of polyribonucleotides and bropirimine on murine transplantable TCC (4, 5). Polyribonucleotides and IFNs have also been effective in inhibiting virus-induced and radiation-induced tumors (27–31). Only one prior study of IFNs in carcinoinduced malignancy has been reported (32). That study, which used an unpurified mouse IFN preparation, demonstrated inhibition of development of s.c. fibrosarcomas after 3-methylcholanthrene administration. Interestingly, a relatively low dose of IFN of 2–10 × 10⁸ units daily was effective (32). Skin papillomas induced by 3-methylcholanthrene or dimethylbenzanthracene have been inhibited by polyribonucleotides (33, 34). Like the inhibition of FANFT carcinomas by bropirimine, administration of polynucleotides late in the evolution of papillomas was ineffective (34). Probing of molecular events in the neoplastic process induced by carcinogens may help dissect mechanisms of antitumor action of IFNs. Efficacy early in the neoplastic process suggests greater effects on initiation and/or microscopic tumor than on cellular proliferation and tumor growth.

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**REFERENCES**


* A. K. Verma and E. C. Borden, unpublished observations.
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